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Effect of Extraction Solvents on Phytochemicals and Antioxidant Potential of Turnip Roots (*Brassica rapa* L.)

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ABSTRACT

Background: *Brassica rapa* (L.) is commonly used as vegetable because of its antioxidant and medicinal properties. Thus, turnip (variety White 4) was characterized by its chemical composition and mineral profile procured from CCS Haryana Agricultural University in Hisar. The effect of solvent on extraction of phytochemicals was investigated.

Methods: Antioxidant potential including total phenolics content (TPC), total flavonoids (TFC), the free radical scavenging activity (DPPH assay) and total antioxidant capacity (TAC) was analyzed.

Result: The aqueous extract contained the highest total phenols (4.56±0.02 mg GAE/g) and flavonoids (2.07±0.05 mg CE/g) as compare to ethanol and acetone extracts. The DPPH free radical scavenging activity of turnip root extracts was highly variable and increased with increasing concentration levels. The aqueous extract showed the best DPPH free radical scavenging activity at the IC_{50} value (1.26 mg/mL) followed by ethanol (11.30 mg/mL) and acetone (12.08 mg/mL) extracts. The total antioxidant capacity was highest in aqueous extract (3.45±0.03) as compared to ethanol (2.95±0.07) and acetone (0.81±0.08 mg AAE/g).

Key words: Antioxidant activity, *Brassica rapa,* Crude fiber, Crude protein, Flavonoids, Minerals, Phenolics.

INTRODUCTION

Recently, the antioxidant activity of many plants and vegetables has been widely studied. Antioxidants from medicinal plants, spicy plants and other plants have been studied to develop natural antioxidant formulations for drugs, cosmetics and other applications. In order to maintain the activity of the plant root and optimize the concentration of known components, phytochemical treatment of plant raw materials is required (Aziz *et al*., 2003). Extraction is an important step in the process of phytochemical treatment to discover bioactive ingredients in plant material (Dhanani *et al*., 2017; Nehra *et al*., 2022). However, the bioactive components of plant extracts are highly dependent on the polarity and pH of the solvent, the particle size of the plant material, the chemistry of the extracted compound, the temperature and the extraction method (Abubaker and Haque, 2020; Devi *et al*., 2020).

Cruciferous vegetables, particularly which are included into the *Brassica* genus, are good sources of a variety of nutrients, antioxidants and health promoting phytochemicals. Main antioxidative components present in *Brassica* vegetables are water soluble which include phenolic compounds (flavonoids) and vitamins (ascorbic acid) while other are lipid soluble such as tocopherols and carotenoids. *Brassica rapa* (L.) commonly known as turnip, is an annual or biennial herbaceous species of the *Brassicaceae* family and in India it is widely cultivated in Bihar, Haryana, Himachal Pradesh, Punjab and Tamil Nadu. The chemical composition of turnip is water, fat, fiber, protein, carbohydrate, Fe, Cu, Zn, Mn (Bangash *et al*., 2011). Turnip root contains a variety of organic compounds with biological activity, such as glucocinolates, phenylpropanoids, flavonoids, phenolics and organic acids (Fernandes, 2007).

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Turnip leaves contains many bioactive organic compounds, such as turnip greens, which have a distinctive bitter and pungent taste related to the content of glucosinolates degradation products (Jones *et al*., 2007). Turnip sprouts are high in glucosinolates, which are sulfur-containing compounds that prevent some types of cancer and provide antifungal, antibacterial and antiparasitic effects. The main isothiocyanates present in turnips are 3-butenyl, 4-pentenyl, β phenylethyl isothiocyanates, which have anti-carcinogenic properties (Zhang and Talalay, 1994). Various field studies have been carried out with different varieties of turnip but a little work has been done on phytochemical studies and antioxidant activity of Turnip (*Brassica rapa* L.) grown at Research Farm of CCS HAU, Hisar. Thus, the main objectives of the study was to evaluate the effect of solvents on the total amount of phenols, flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity and total antioxidant capacity by modified phosphomolybdenum assay.

MATERIALS AND METHODS

Collection of experimental material

Fresh, fully mature turnips (Turnip variety White 4) were procured from research farm in the Department of Vegetable Science, CCS Haryana Agricultural University, Hisar. Turnip roots were peeled, sliced, dried at room temperature then placed in drying oven to prepare turnip extracts and powders. Similarly, all chemicals and standards were purchased from Sigma- Aldrich and Merck. Experiments were carried out in Department of Chemistry, CCS HAU, Hisar during the period of 2017.

Proximate composition analysis

Proximate analysis of turnip roots was carried out for moisture content, crude fibre (Maynard, 1970), crude protein according to the standard methods as described in Association of Official Analytical Chemists (AOAC, 1975). Total sugars were estimated by the modified method of Dubois *et al*., 1956. Reducing sugar was estimated by the method of Nelson, (1944) as modified by Somogyi, (1952). The content of non-reducing sugar was calculated as the difference between the content of total sugar and reducing sugar.

Estimation of minerals content

Minerals were estimated by Jackson, (1973) and Ruig *et al*. (1986). Two gram powdered sample of turnip roots was digested with 15 mL of diacid mixture ($4HNO_3$: $1HClO_4$) in a conical flask by heating on hot plate in open space till clear white precipitates settled down at bottom of conical flask. The precipitates were dissolved in 1% HCl prepared in double glass distilled water, filtered and final volume of filtrate was made up to 50 mL with double distilled water.

Extraction of phytochemicals

Ten gram of turnip powder sample was placed in a filter paper thimble and extracted with a classic soxhlet apparatus in 150 mL of solvent (acetone, ethanol and water). After the primary extraction was completed, the thimble residue was extracted twice using appropriate amounts of each solvent. Filtrates of each solvent obtained in the three extraction steps were collected and the volume was recorded. The extracts were used to measure their phytochemicals and antioxidant capacity. Total phenolics content (TPC), total flavonoids (TFC), DPPH radical scavenging activity (1, 1-diphenyl-2-picrylhydrazyl) and TAC (Total antioxidant capacity) tests were performed to assess antioxidant perspectives.

Estimation of total phenolics

The total phenolics content was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Accordingly, 0.2 mL extract fraction was mixed with 1.0 mL of 1 mol/L Folin-Ciocalteu reagent. Then 2.0 mL of sodium carbonate (20%, w/v) was added, the solutions were mixed and the volume was made 10.0 mL with water. After 8 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes and then the absorbance of the supernatant was measured at 730 nm using a UV-VIS Double Beam Spectrophotometer (Shimadzu, UV 1900). A calibration curve was created using gallic acid as standard and results are expressed in milligrams of gallic acid equivalent per gram (mg GAE/g).

Estimation of flavonoids

The flavonoids content was determined by aluminium chloride colorimetric assay (Ribarova and Atanassova, 2005). For this purpose, 1.0 mL of extract, 4.0 mL of double distilled water and 0.3 mL of $NaNO_2$ (5%, w/v) were added. After 5 min, 0.3 mL of $AlCl_3$ (10%, w/v) was added. Immediately, 2.0 mL of 1M NaOH was added and the total volume was made up to 10.0 mL with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-VIS Double Beam Spectrophotometer (Shimadzu, UV 1900). The calibration curve was prepared using catechin as standard and results are expressed as mg catechin equivalents per gram (mg CE/g).

Estimation of DPPH free radical scavenging activity

The antioxidant activity of the extracts was evaluated by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method[18]. Acetone, ethanol and aqueous extracts of turnip root powder were dried and the weight of the dried mass was recorded. A stock solution (50 mg/mL) was made by re-dissolving the dry mass of the acetone and ethanol extracts in an appropriate amount of methanol. For evaluation of antioxidant activity, 3.0 mL of 2,2'diphenyl1 picrylhydrazyl radical (DPPH; 0.1 mM in100% methanol) was added to 0.2 mL of extracts (various concentrations) and mixed thoroughly for 5 minutes. For the antioxidant activity ofthe water extracts (various concentrations), a stock solution of DPPH was prepared with 50% (v / v) methanol: water and the rest of the procedure was the same. A control group containing 0.2 mL of each solvent was also prepared instead of the extract. Absorbance of samples and controls was measured at 517 nm after 30 minutes incubation in a darkroom at room temperature using UV-VIS Double Beam Spectrophotometer (Shimadzu, UV 1900) for blanks containing their respective solvents in 3 replicates. Graphs were drawn by plotting the percentage DPPH free radical trapping activity (y-axis) versus extract concentration (x-axis). The following is a quadratic regression equation ($y = ax^2 + bx + c$) obtained using Microsoft Excel software and calculated using the quadratic equation IC_{50}. The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ = The absorbance of control.

A_{sample} = The absorbance of the sample.

Estimation of total antioxidant activity

Estimate the total antioxidant capacity extracts of turnip roots by the modified phosphomolybdenum method (Prieto *et al*., 1999). In a glass vials, 0.3 ml of each extract was placed

and 3 ml of phosphorus molybdenum reagent was added, the solution was mixed well and capped. These vials were incubated for 90 min at 95°C. After this, the contents of the vials were cooled and absorbance was measured at 695 nm on blanks prepared on a UV-VIS Double Beam Spectrophotometer (Shimadzu, UV 1900) against a blank prepared. The total antioxidant capacity was calculated in aqueous extracts from the standard curve and expressed as mg AAE/g.

All experiments were performed in triplicate for statistical study and are expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed to assess significant differences between the means values of samples in online statistical analysis (OPSTAT). IC_{50} values of DPPH free radical scavenging activity was calculated using a quadratic regression equations (Table 1). The correlation between of total phenolics, total flavonoids and DPPH free radical trapping IC_{50} values with total antioxidant capacity was determined using the Karl Pearson method in Microsoft Excel and all other measurements were also performed in Microsoft Excel 2019.

RESULTS AND DISCUSSION

Composition profiling and mineral content

The nutritional content of turnip roots was determined by proximity analysis.In the present investigation moisture, crude fiber, crude protein, total sugar, reducing and non reducing sugars were determined in turnip roots at level of 4.68±0.07%, 10.34±0.10%, 13.39±0.36%,169.11±0.80, 159.70±0.88, 9.41±0.38 respectively (Table 1). The results obtained for compositional analyzes are comparable with Saeed *et al*. (2012); Atta *et al*. (2017); Azizuddin and Ghafoor, (2016) who found similar results for moisture, fibre, protein, total sugars, reducing and non reducing in turnip roots.In the current study, iron, copper, zinc and manganese were present on a dry weight basis in appreciable amounts 39.26, 5.83, 12.44 and 93.60 ppm, respectively in turnip roots (Table 1). However, finding of Gutierrez *et al*. (2008) showed some variations with current study regarding iron 140 ppm, copper 6.7 ppm and zinc 22.20 ppm.

Total phenolic content

The total phenolic content (TPC) in turnip root extracts varied widely in three solvents. On a dry weight basis, the aqueous extract of turnip contained the highest total phenolics content, namely 4.56 mg GAE/g, followed by 3.75 mg GAE/g in the ethanol extract and 1.99 mg GAE/g in the acetone extract. Our results are consistent with other studies reporting 0.17-0.70 mg GAE/g (fresh weight) of total phenols in root extracts of seven genotypes of turnip (Sengul *et al*., 2011). The water extract of Rang Chuet was reported to have the highest phenol content, followed by ethanol and acetone extracts thus showing the effect of extracting solvents on phytochemicals (Oonsiviali *et al*., 2008). Total phenolics content was found to be 5.64 mg/g in fresh roots juice extract of turnip (Anitha and Dharsini, 2014).

Table 1: Proximate composition and mineral profile of turnip roots.

Parameters	Results
Moisture content (%)	4.68 ±0.07
Crude fiber (%)	10.34±0.10
Crude protein (%)	13.39±0.36
Total sugars (mg/g)	169.11±0.80
Reducing sugars (mg/g)	159.70±0.88
Non reducing sugars (mg/g)	9.41±0.38
Fe (ppm)	39.26±0.08
Mn (ppm)	93.60±0.29
Zn (ppm)	12.44±0.04
Cu (ppm)	5.83±0.05

Table 2: Total phenolics and flavonoids content of turnip extracts prepared using different solvents.

Solvent	Total phenolics content (mg GAE/g)	Flavonoids content (mg CE/g)
Acetone	1.99±0.03	0.59±0.01
Ethanol	3.75±0.01	0.79±0.01
Aqueous	4.56±0.02	2.07±0.00

Total flavonoids content

The flavonoid content of the turnip extract was very diverse in the three solvents (Table 2). On a dry weight basis, the aqueous extract of turnip contains the highest flavonoid content *i.e*. 2.07 mg CE/g, 0.79 mg CE/g in ethanol extract, 0.59 mg CE /g in acetone extract. Our results are consistent with other studies reporting that flavonoid content varies between 0.7-7.6 mg CE/g of turnip roots (Aires *et al*., 2011).

DPPH free radical scavenging activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical (purple) and is widely used as a measure of the electron donating ability of antioxidants because it changes to its non-radical form (yellow) by withdrawing one electron during experimental conditions. The present study showed that the DPPH free radical scavenging activity (%) of acetone, ethanol and aqueous extracts of turnips differed significantly and increased with increasing concentration levels, as shown in Table 3. Antioxidant activity (%) was found to be varied from 38.10 to 75.32 % for aqueous extract (at concentration levels 1.0 to 5.0 mg/mL), 17.42 to 80.59% for ethanolic extract and from 12.33 to 80.43% for acetone extract at different concentrations ranging 1.0 to 50.0 mg/mL of the extracts. The IC_{50} value of aqueous extract was lowest (1.26 mg/mL) showing that aqueous extract has highest antioxidant activity followed by ethanol and acetone extracts. The results of this study are consistent with macadamia studies showing that absolute methanol extract has the highest antioxidant activity after water, ethanol, acetone or acetonitrile extraction (Dailey and Vuong, 2015). Antioxidant activity in roots extract of *Brassica rapa* has been reported to be ranged from 18 to 72% at concentrations level ranging from 0.5 to 3.0 mg/mL (Saeed *et al*., 2012).

Table 3: DPPH free radical scavenging activity (%) and IC_{50} value (µg/mL) of different extracts of turnip.

Extracts	DPPH free radical scavenging activity at different concentration (mg/mL)						IC_{50} (mg/mL)
	50.0	25.0	10.0	5.0	2.5	1.0	
Acetone	80.43	74.65	46.31	30.52	18.11	12.33	12.08
Ethanol	80.59	76.61	48.29	29.76	23.48	17.42	11.30
Aqueous	a	a	a	75.32	69.10	38.10	1.26

'a' represent absent.

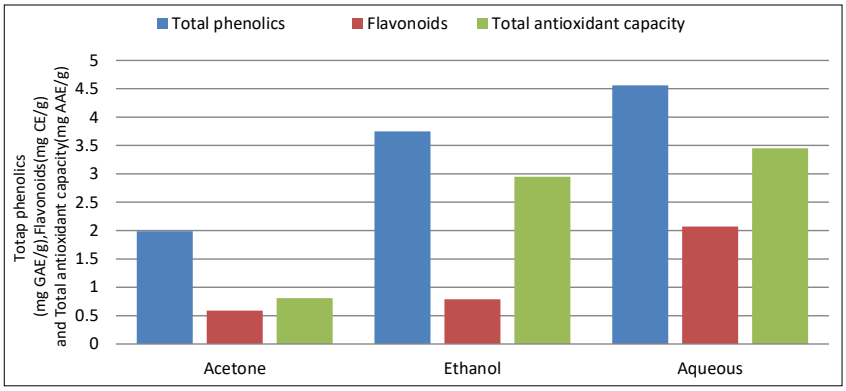


Fig 1: Effect of extraction solvents on total phenolics, flavonoids and total antioxidant capacity of Turnip roots.

Total antioxidant capacity

The phytochemical components present in the extract contributed to the major antioxidant activity. High phenol content plays a role in most plant materials as antioxidants. The total antioxidant capacity was calculated in acetone, ethanol and aqueous extracts from the standard curve and expressed as mg AAE/g. The total antioxidant capacity was highest in an aqueous extract (3.45±0.03 mg AAE /g), then in ethanol extract (2.95± 0.07 mg AAE /g) and acetone extract (0.81±0.08 mg AAE / g). Effect of Extraction Solvents on total phenolics, flavonoids and total antioxidant capacity of Turnip roots are shown in Fig 1. This linear relation of antioxidant activity with total phenolic content and total flavonoid content was also confirmed by previous findings (Wan *et al*., 2011).

CONCLUSION

The turnip root powder is a source of abundant nutrients and bioactive compounds. The results of this study show that solvent play an important role in the extraction of phytochemicals and the evaluation of antioxidant activity. The aqueous extract of turnip had the highest total phenol and flavonoid content and the highest antioxidant activity compared to the ethanol and acetone extracts.

Conflict of interest

I confirm that there is no conflict of interest to declare on behalf of all authors for this manuscript.

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